DOI: 10.2428/ecea.2018.25(1)10 ECOL CHEM ENG A. 2018;25(1):93-105

Małgorzata NABRDALIK

PRODUCTION OF INDOLE ACETIC ACID BY ENDOPHYTIC *Bacillus* STRAINS

PRODUKCJA KWASU INDOLILOOCTOWEGO PRZEZ ENDOFITYCZNE SZCZEPY *Bacillus*

Abstract: The objectives of this study were to isolate and characterize endophytic bacteria from sugar beet roots focusing on their ability to produce indole acetic acid (IAA). In order to isolate endophytic bacteria the sugar beet roots were used. To determine the amounts of IAA produced by endophytic *Bacillus* strains (*B. amyloliquefaciens*, *B. megaterium* and *B. subtilis*), a colorimetric technique was applied with Salkowski reagent. The isolates were grown in Laurin Broth medium supplemented with L-tryptophan over the concentration range of 100–10000 μg/cm^3 and incubated at 30 ^{o}C for 7 days. The highest concentration of IAA was recorded after 4 days of culturing in the supernatant obtained from the media containing 10000 μg/cm^3 of tryptophan. For the strain *B. subtilis* and *B. megaterium* the concentration of IAA marked in the post –culturing liquid amounted about 82.00 μg/cm^3, and for the *B. amyloliquefaciens* strain it amounted over 121.28 μg/cm^3. The strains *Bacillus* under study produced IAA in a different amount in the presence of L-tryptophan and in its absence.

Keywords: *Bacillus,* endophytes, tryptophan, auxins

Introduction

Endophytes belong to the microorganisms that have attracted researchers' attention in recent years. They derive mainly from rhizosphere and colonize internal plant tissues without causing disease symptoms [1, 2]. Most of the known endophytes belong to the genera *Aerobacter*, *Aeromonas*, *Bacillus*, *Curtobacterium*, *Enterobacter*, *Pseudomonas*, *Erwinia* and *Microbacterium* [3–5]. They help plants adapt to unfavourable environmental conditions such as excessive salinity, lack of water, stress caused by pesticides, heavy metals or hydrocarbons. Endophytic bacteria are particularly beneficial in the process of plants growth and development and thus are applied in the process of biological plant protection and increasing plant yield.

[1] Independent Chair of Biotechnology and Molecular Biology, University of Opole, ul. kard. B. Kominka 6, 45-032 Opole, Poland, phone: +48 77 401 60 56, email: mnabrdalik@uni.opole.pl

In the colonization of plants by bacteria, it is important that bacteria have the ability to produce enzymes degrading the plant cell wall (CWDE, Cell Wall Degrading Enzymes) at a significantly lower level when compared to phytopathogenic microorganisms [3]. The endophytes possess the ability to bind nitrogen, produce phytohormones, synthesize 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) and increase the bioavailability of phosphorus. They are also responsible for the production of hydrogen cyanide, ammonia, siderophores, antimicrobial compounds or biosurfactants. The strains producing plant hormones of auxin class, i.e. indole-3-acetic acid (IAA) are of particular interest. IAA, produced by endophytic microorganisms is probably involved in establishing contact between the bacteria and the plant and plays a significant role in the development of the root plant system. Low levels of IAA stimulate root elongation, while higher affects the formation of lateral and adventitious roots. However, an application of high concentrations of IAA induces ethylene production which inhibits the plant growth [6–9]. In addition, the bacterial IAA increases the amount of polysaccharides produced by the plant that can be used by other microorganisms. This promotes further colonization of plants by different potential endophytes and the development of rhizodermal bacteria [1, 10].

There have been identified several pathways of IAA synthesis in endophytic bacterial cells. Its main precursor is tryptophan. Symbiothic bacteria produce IAA as indolyl--3-pyruvic acid (IPyA indole-3-pyruvate path), whereas phytopathogenic strains produce IAA from indolyl-3-acetamide (IAM indole-3-acetamide pathway) [11–13].

The aim of this paper was to isolate endophytic bacteria from the sugar beetroot and identify their abilities to produce indole-3-acetic acid (IAA).

Materials and methods

Isolation and identification of endophytic bacteria

In order to isolate endophytic bacteria sugar beet roots were used. They were thoroughly washed and fragments of tissues for tests were cut out under aseptic conditions. Obtained samples were then rinsed with 70 % propanol, shaken for 5 minutes in sodium hypochloride solution and rinsed with sterile water. In order to isolate endophytic bacterial cells, tissues were macerated in phosphate-buffered saline (PBS). The tissue homogenate was applied onto tryptone soya agar (TSA, BioMaxima) after short pasteurization. The growing media were incubated at 28 ^{o}C for 48 hours. The preliminary identification of isolated mono bacterial cultures was carried out on the basis of microscopic description, biochemical properties and diagnostic test Api 50CHB (BioMerieux).

The isolated bacteria, recognized as *Bacillus* spp., were identified as species with 16S rDNA sequencing procedure. The amplification was carried out with the following primers: 27F (5'-AGAGTTTGATCTTGGCTCAG-3') and 1492R (5'-ACGGTTACC TTGTTACGACTT-3') and with the use of Direct PCR Kits – Terra PCR Direct Polymerase Mix (Clontech, Mountain View, CA, USA = Takara Bio USA). Terra PCR Direct enables to skip DNA extraction and purification stage, and proceed directly with

PCR amplification. PCR reaction was conducted in 50 mm^3 capacity with 5 mm^3 of sample (1-day-old cultures), 25 mm^3 2X Terra PCR Direct Buffer (with Mg^{2+}, dNTP), 15 pmoles of forward and reverse primer mixture and 1 mm^3 Terra PCR Direct Polymerase Mix (1.25 U/ mm^3). The PCR reaction was performed with initial denaturation at 98 ^oC for 2 minutes followed by 40 cycles with denaturation for 10 seconds at 98 ^oC, annealing for 15 seconds at 60 ^oC and extension at 68 ^oC for 60 seconds for each kb fragment. The amplified products were separated by electrophoresis with a 2.0 % agarose gel and stained with ethidium bromide. Detection of PCR products was determined for ethidium bromide stained agarose gel (2.0 % in TBE buffer) using Gel-doc (Bio-Rad). The PCR samples were sent for sequencing at Genomed (www.genomed.pl). The sequences identity were compared with those present in GenBank database (www.ncbi.nlm.nih.gov/BLAST/) using the BLASTN algorithm.

Screening of bacterial strains for hydrolytic enzymes production

Production of CWDE (protease, α-amylase, lipase and cellulase) is a common mechanism used by bacteria to inhibit the growth of other microorganisms.

For determining the production of protease, α-amylase, lipase and cellulase 10 mm^3 of the bacterial cells suspension was streaked on respective agar medium. The plates were incubated at 28 ^oC for 7 days.

In order to determine the production of protease, skim milk agar plates were used and after the incubation time the plates were observed for clear zones around the colony [14].

To detect the production of α-amylase, 1.0 % iodine solution was poured into the starch agar after the incubation and a yellow zone around the colony was observed against the dark blue back ground [15].

Lipase production was proved by supplementing the nutrient agar with 1.0 % tributyrin (Sigma-Aldrich). Appearance of the clear zone around the colony indicated occurrence of extracellular lipase production.

Cellulase production was determined by using media with 1.0 % carboxymethylcellulose CMC (Sigma-Aldrich) in basal medium [14]. After cell growth, the presence of extracellular cellulase was detected by flooding the plates with 0.1 % Congo red solution for 15 minutes and decolouring them with 1.0 % NaCl solution for 5 minutes. A clear zone against the red back ground indicated that the bacteria was positive for cellulase production.

Screening of bacterial strains for plant growth promoting traits

Siderophore production

The isolates were checked for the production of siderophores on CAS blue agar medium containing complex chrome azurol S (CAS/Fe^{+3}/hexadecyltrimethylammonium bromide) [16]. The bacterial strains were inoculated on the CAS agar plates and

incubated at 28 °C for 48 h. Development of orange halos around the colonies were taken as the measurement of siderophore production.

Hydrogen cyanide (HCN) production

Production of HCN was assessed by growing the bacteria in tryptone soya agar (TSA) supplemented with glycine (4.4 g/dm^3). Filter paper soaked in picric acid and Na_2CO_3 solution was fixed to the underside of the lids of plates and incubated for 7 days at 28 °C. A change in the filter paper color from deep yellow to orange brown to dark brown was considered to be the indication of HCN production [17].

Ammonia production

The bacterial isolates were tested for the production of ammonia in peptone broth. The strains were inoculated into peptone broth and incubated at 28 °C for 48 h. After incubation Nessler's reagent (0.5 cm^3) was added to each tube. The formation of brown to yellow precipitate was considered to be a positive test for ammonia production [15].

Phosphate solubilization

The bacterial isolates were screened for phosphate solubilization using Pikovskaya medium [18]. The media inoculated with the isolates were incubated at 28 °C for 7 days. The plates were observed for the formation of clear zone around the colony, which indicated solubilization of phosphate present in the medium.

IAA production characteristic

To determine the amounts of IAA produced by endophytic bacteria strains, a colorimetric technique was applied with Salkowski reagent [19].

The isolates of *Bacillus* spp. were grown in LB medium (Laurin Broth) supplemented with L-tryptophan at concentrations of 100, 500, 1000, 5000 and 10000 μg/cm^3 and incubated at 30 °C for 7 days. In the control trials the bacterial cultures were grown in the medium without L-tryptophan. The production of IAA was measured after every 24 hours. After the incubation, the cells were centrifuged (10000 rpm for 20 minutes at 10 °C) and 2 cm^3 of supernatant was mixed with 4 cm^3 of Salkowski's reagent and kept in the dark at room temperature. The optical density (OD) was recorded at 530 nm after 30 minutes. The quantification of IAA was carried out using a standard curve with known concentrations of pure IAA (Sigma-Aldrich), prepared separately. Values are expressed in μg/cm^3.

Statistical analysis

Statistical significance was determined using an analysis of variance (ANOVA) followed by Tukey's HSD test. Values were considered significantly different at $p < 0.05$.

Results and discussion

A total of three different bacterial strains were isolated after pasteurization from the sugar beet roots on the basis of colony morphology and colors. All the isolates were Gram-positive and were able to form endospores. The preliminary identification performed with tests Api 50CHB determined biochemical properties of bacteria and thus classify them as *Bacillus.* In enzymatic tests, the strains used different carbohydrates: glycerol, L-arabinose, D-ribose, D-xylose, glucose, fructose, mannose, inositol, mannitol, sorbitol, methyl-D-glucoside, arbutine, esculine, salacine, cellobiose, maltose, saccharose, trehalose, inuline, raffinose, amidon, glycogene, gentiobiose and turanose and additionally one of the following: galactose, N-acetyl-glucosamine, amygdaline, lactose, melibiose and melezitose (Table 1). On the basis of their morphology, biochemical characteristics and 16S rDNA gene sequence isolates were identified as *Bacillus amyloliquefaciens*, *B. megaterium* and *B. subtilis*.

Table 1

Biochemical characterization of endophytic *Bacillus* strains of sugar beet roots

Test	*Bacillus amyloliquefaciens*	*Bacillus megaterium*	*Bacillus subtilis*
Glycerol	+	+	+
Erytheritol	–	–	–
D-Arabinose	–	–	–
L-Arabinose	+	+	+
D-Ribose	+	+	+
D-Xylose	+	+	+
L-Xylose	–	–	–
Adonitol	–	–	–
Methyl-β-D-Xylopyranoside	–	–	–
Galactose	–	–	+
D-Glucose	+	+	+
D-Fructose	+	+	+
D-Mannose	+	+	–
L-Sorbose	–	–	–
Rhamnose	–	–	–
Dulcitol	–	–	–
Inositol	+	+	+
Mannitol	+	+	+
Sorbitol	+	+	–
α-Methyl-D-Mannoside	–	–	–
α-Methyl-D-Glucoside	+	+	–
N-Acetyl-Glucoside	–	–	+
Amygdaline	–	–	+
Arbutine	+	+	+
Esculine	+	+	+

Table 1 contd.

Test	*Bacillus amyloliquefaciens*	*Bacillus megaterium*	*Bacillus subtilis*
Salicine	+	+	+
Cellobiose	+	+	+
Maltose	+	+	+
Lactose	–	–	+
Melibiose	–	–	+
Saccharose	+	+	+
Trehalose	+	+	+
Inuline	–	+	+
Melezitose	–	–	–
D-Raffinose	+	+	+
Amidon	+	+	+
Glycogene	+	+	+
Xylitol	–	–	–
β-Gentiobiose	–	–	+
D-Turanose	+	+	+
D-Lyxose	+	+	–
D-Tagatose	–	–	–
D-Fucose	–	–	–
L-Fucose	–	–	–
D-Arabitol	–	–	–
L-Arabitol	–	–	–
Gluconate	–	–	–
2-Keto-Gluconate	–	–	–
5-Keto-Gluconate	–	–	–

"+" – presence of activity; "–" – absence of activity.

With regard to production of hydrolytic enzymes, all the endophytic bacterial strains were positive for protease, amylase and lipase. Only *B. megaterium* was negative for cellulose production (Table 2).

Table 2

Production of hydrolytic enzymes by endophytic *Bacillus* strains

Production of	*Bacillus amyloliquefaciens*	*Bacillus megaterium*	*Bacillus subtilis*
α-amylase	+	+	+
Protease	+	+	+
Lipase	+	+	+
Cellulase	+	–	+

"+" – presence of activity; "–" – absence of activity.

There are many reports on the production of hydrolytic enzymes by endophytic bacteria [20–22]. Production of extracellular hydrolytic enzymes by microorganisms plays an important role, for example in the management of plant pathogens. They also appear to be important for the colonization of plant roots. In authors' own studies, Bacillus strains showed high activity of lytic enzymes involved in the degradation of the cell wall. However, Cho et al. [14] suggested that hydrolytic enzymes can be produced by endophytes only in the early stage of invasion, and not in the phase when the plant tissue is infected. Moreover, the authors, on the basis of 16S rRNA gene sequence analysis, proved that the majority of Gram-positive endophytic bacteria with low content of G+C show cellulolytic activity, whereas some Gram-positive bacteria with high G+C content show only protease activity and no cellulolytic activity. Cho et al. [14] also proved lack of any cellulolytic activity for *B. megaterium*. Hence the differences in activity between the analyzed *Bacillus* strains and the research data published by other researchers [21, 22].

All isolates were tested for plant growth promotion traits by qualitative determination of production of siderophore, hydrogen cyanide, ammonia, phosphate solubilization and auxin production. None of the isolated *Bacillus* strains was able to produce siderophore but they were able to produce IAA. Only *B. megaterium* was positive for HCN production and *B. subtilis* was able to produce ammonia and solubilize inorganic phosphate (Table 3).

Table 3

Plant growth promoting attributes of endophytic *Bacillus* strains

Production of	*Bacillus amyloliquefaciens*	*Bacillus megaterium*	*Bacillus subtilis*
Siderophore	–	–	–
HCN	–	+	–
Ammonia	–	–	+
Phosphate solubilization	–	–	+
Indole-3-acetic acid	+	+	+

"+" – presence of activity; "–" – absence of activity.

The following research concerned the evaluation of the ability of tested *Bacillus* strains to synthesize indole-3-acetic acid. It has been determined how the concentration of L-tryptophan, an auxin precursor, and the time affected the amount of produced IAA. Its presence was noted in post-culturing liquid of all three tested strains, both in the presence and absence of L-tryptophan (Fig. 1–3). The highest amount of IAA was produced by tested strains on the fourth day of culturing in the presence of 10.000 μg/cm^3 of L-tryptophan. The most active strain under these conditions was *B. amyloliquefaciens* producing 121.3 μg/cm^3 of IAA. The lowest concentration of IAA was recorded, in the first three days of the experiment, for the growing medium without the amino acid and for the medium containing its lowest amount. The concentration of IAA amounted circa 20.0 μg/cm^3 and the differences between noted results were statistically insignificant. In the remaining variants of the experiment, differences in the

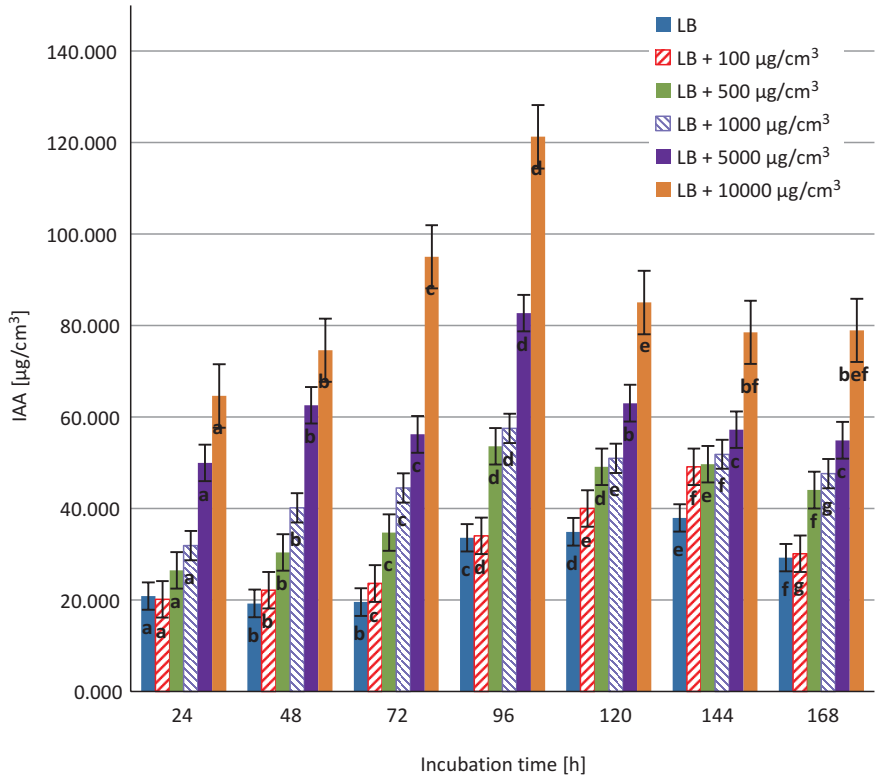

Fig. 1. Effect of L-tryptophan on IAA production by *Bacillus amyloliquefaciens*. Different letters indicate significant differences (ANOVA, $p < 0.05$, Tukey's HSD test)

amount of produced IAA were statistically significant. After four days of incubation, a slow decrease in the amount of the synthesized IAA by *B. amyloliquefaciens* was noted (Fig. 1). The other strains, *B. megaterium* and *B. subtilis* produced IAA at a similar level, however, significantly lower when compared with *B. amyloliquefaciens*. The two strains, produced the highest amounts of IAA on the fourth day in the presence of the highest concentration of amino acid in the medium, and amounted 82.58 and 81.13 µg/cm^3 of IAA, respectively (Fig. 2–3). Increasing the incubation time to 7 days resulted in a statistically significant decrease in the production of IAA. The amount of produced IAA was growing with increasing incubation time only in case of supernates obtained from the culturing medium containing 1.000 µg/cm^3 of L-tryptophan.

Bacterial endophytes can promote plant growth directly by producing compounds enabling plants to absorb nutrients from the environment. The mechanisms include: binding biological nitrogen, hydrogen cyanide production, solubilization of phosphorus, siderophore production and the biosynthesis of phytohormones [23, 24].

In authors' research, none of tested strains showed the ability to promote the plant growth by performing all of the above mentioned mechanisms. Also in Arruda et al. [16] research only 25 % isolates demonstrated all of the characteristics evaluated and

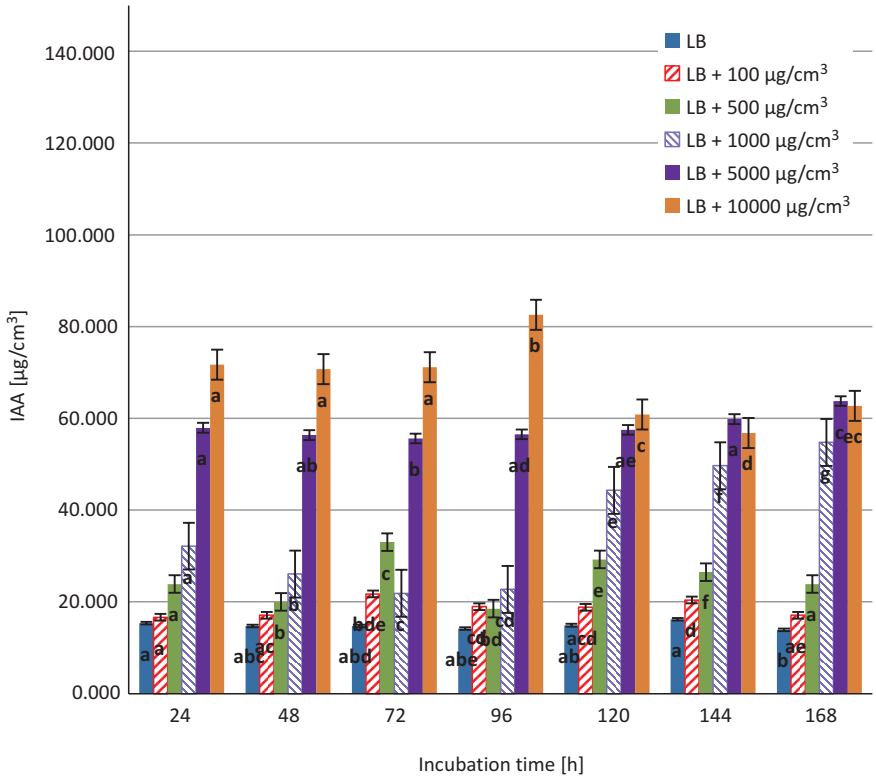

Fig. 2. Effect of L-tryptophan on IAA production by *Bacillus megaterium*. Different letters indicate significant differences (ANOVA, $p < 0.05$, Tukey's HSD test)

42 % isolates possessed two characteristics: to produce indole-acetic acid and to solubilize phosphates, or produce indole-acetic acid and siderophores. However, none of the strains showed simultaneous phosphate solubilization and siderophore production. They found rhizobacteria producing siderophores which belonged to the genera *Pseudomonas* and *Burkholderia*. Many studies have reported that *Pseudomonas* spp. are potent siderophore producers [9, 25]. Zhao et al. [26] presented different results when compared with the results obtained by the authors. Most of the endophytic *Bacillus* strains under study were able to produce siderophores and solubilize phosphates. Whereas, Kumar et al. [27] noted that all four *Bacillus* strains tested in their research synthesized IAA, three solubilized phosphates and only one produced siderophores. A large group of endophytes is capable of solubilizing inorganic phosphate in soil which is determined by their ability to produce organic acids with carboxyl groups able to chelate cations, especially cations P and Ca and thus help plants assimilate soluble P [16, 26]. Siderophores play an important role in promoting plant growth employing two mechanisms, direct by supplying Fe to the plant or by limiting the availability of Fe to pathogens [26]. However, in presented research, only one of the isolates was able to solubilize phosphates and none produced siderophores.

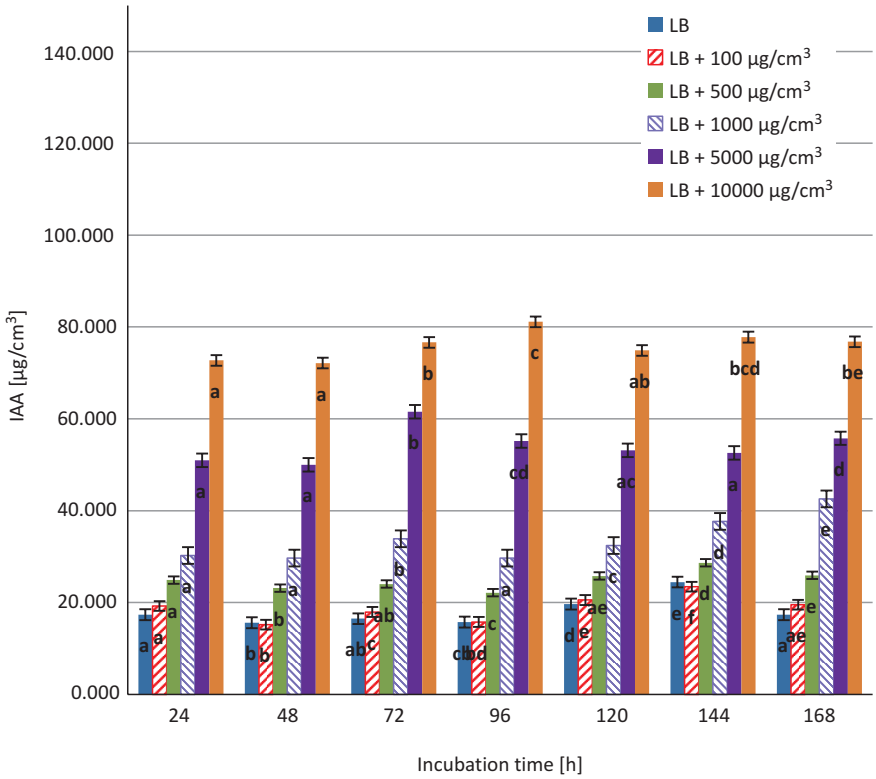

Fig. 3. Effect of L-tryptophan on IAA production by *Bacillus subtilis*. Different letters indicate significant differences (ANOVA, $p < 0.05$, Tukey's HSD test)

The most common growth regulator produced by endophytic bacteria is indole--3-acetic acid, which might help with nitrogen binding from the atmosphere and which plays an important role in the growth and development of roots. In authors' own research presented in this paper, all tested strains of *Bacillus* produced indole-3-acetic acid. The analysis of available reference data enables to conclude that most endophytes (98 %) are able to produce IAA in the presence of tryptophan, assuming that noted value of IAA amounting 0.1 µg/cm^3 or higher is a confirmation of IAA production [16]. The amount of synthesized IAA varies and depends mainly on applied amount of tryptophan. Zhao et al. [26] in their research noted IAA production by all endophytic *Bacillus* strains and the amount ranged from 11.5 µg/cm^3 (for *B. megaterium*) to 22.9 µg/cm^3 (for *B. subtilis*) after 72 h of incubation on the medium containing 100 µg/cm^3 of L-tryptophan. An application of a 10-fold higher concentration of L-tryptophan resulted in different amounts of IAA produced by *B. subtilis* obtained by different authors, e.g. 24.13 and 2.10 µg/cm^3 of IAA [19], 8.80 µg/cm^3 of IAA [28] or 10.62 µg/cm^3 of IAA [29]. Taking into account the above mentioned data, the amount of IAA produced by endophytic bacteria can vary between different species and strains, and depends on the culturing conditions, the growth phase and the availability of the

substrate. Moreover, the differences in the efficiency of IAA production by endophytes can also be connected with individual properties of each strain. Endophytic bacteria utilize different pathways of the IAA biosynthesis, and single bacterial strain sometimes encompasses more than one pathway [11, 13, 18].

Conclusions

Conducted research evaluated endophytic strains belonging to the genus *Bacillus* in terms of their ability to produce IAA depending on the time and the concentration of the substrate. It has been noticed that biosynthesis of IAA, the dose of L-tryptophan and an incubation time is directly proportional. During the first four days of culturing, the increase in L-tryptophan concentration contributed to an increase in the production of IAA. The maximum amount of IAA was produced by *Bacillus* strains after four days of culturing in the presence of 10,000 $\mu g/cm^3$ of L-tryptophan. Among tested strains, endophytic strains of *B. subtilis* showed the activity of enzymes degrading the plant cell wall and employed inorganic phosphate solubilization as one of the major mechanisms of plant growth promotion. Therefore, it can be applied as a factor promoting plant growth in agriculture to increase plant growth.

References

[1] Pinski A, Betekhtin A, Hupert-Kocurek K, Mur LAJ, Hasterok R. Defining the Genetic Basis of Plant – Endophytic Bacteria Interactions. Int J Mol Sci. 2019;20(8):1947. DOI: 10.3390/ijms20081947.

[2] Mastretta C, Barac T, Vangronsveld J, Newman L, Taghavi S, Van der Lelie D. Endophytic Bacteria and their Potential Application to Improve the Phytoremediation of Contaminated Environments. Biotechnol Genet Eng Rev. 2006;23:175-207. DOI: 10.1080/02648725.2006.10648084.

[3] Pawlik M, Płociniczak T, Piotrowska-Seget Z. Bakterie endofityczne i ich znaczenie w mikrobiologii środowiskowej, medycynie i przemyśle (Endophytic bacteria and their role in environmental microbiology, medicine and industry). Post Mikrobiol. 2015;54(2):115-122. http://www.pm.microbiology.pl/web/archiwum/vol5422015115.pdf.

[4] Gouda S, Das G, Sen KS, Shin HS, Patra KJ. Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. Front Microbiol. 2016;7:1538. DOI: 10.3389/fmicb.2016.01538.

[5] Nabrdalik M, Moliszewska E. Wykorzystanie antagonistycznych właściwości Bacillus subtilis wobec Rhizoctonia solani (Application of antagonistic properties of *Bacillus subtilis* against *Rhizoctonia solani)*. Proc ECOpole. 2017;11(1):231-240. DOI: 10.2429/proc.2017.11(1)025.

[6] Overvoorde P, Fukaki H, Beeckman T. Auxin Control of Root Development. Cold Spring Harb Perspect Biol. 2010;2:a001537. DOI: 10.1101/cshperspect.a001537.

[7] Glick BR. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica. 2012;1-15. http://dx/doi.org/10.6064/2012/963401.

[8] Li J, Ovakim DH, Charles TC, Glick BR. An ACC deaminase minus mutant of Enterobacter cloacae UW4 no longer promotes root elongation. Curr Microbiol. 2000;41:101-105. DOI: 10.1007/s002840010101.

[9] Patten CL, Glick BR. Role of *Pseudomonas putida* Indoleacetic Acid in Development of the Host Plant Root System. Appl Environ Microbiol. 2002;68:3795-3801. DOI: 10.1128/AEM.68.8.3795-3801.2002.

[10] Liu H, Carvalhais LC, Crawford M, Singh E, Dennis PG, Pieterse CMJ, Schenk PM.Inner Plant Values: Diversity, Colonization and Benefits from Endophytic Bacteria. Front Microbiol.2017;8:2552. DOI: 10.3389/fmicb.2017.02552.

[11] Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev. 2007;31:425-448. DOI: 10.1111/j.1574-6976.2007.00072.x.

[12] Duca D, Lorv J, Patten CL, Rose D, Glick BR. Indole-3-acetic acid in plant–microbe interactions. Antonie van Leeuwenhoek. 2014;106:85-125. DOI: 10.1007/s10482-013-0095-y.

[13] Camerini S, Senatore B, Lonardo E, Imperlini E, Bianco C, Moschetti G, Rotino GL, Campion B, Defez R. Introduction of a novel pathway for IAA biosynthesis to rhizobia alters vetch root nodule development. Arch Microbiol. 2008;190:67-77. DOI: 10.1007/s00203-008-0365-7.

[14] Cho KM, Hong SY, Lee SM, Kim YH, Kahng GG, Lim YP, Kim H, Yun HD. Endophytic Bacterial Communities in Ginseng and their Antifungal Activity Against Pathogens. Microbial Ecol. 2007;54(2):341-351. DOI: 10.1007/s00248-007-9208-3.

[15] Fouda AH, Hassan SED, Eid AM, Ewais EED. Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). Annals AgricultSci. 2015;60(1):95-104. https://doi.org/10.1016/j.aoas.2015.04.001.

[16] Arruda L, Beneduzi A, Martins A, Lisboa B, Lopes C, Bertolo F, Passaglia LMP, Vargas LK. Screening of rhizobacteria isolated from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. Appl Soil Ecol. 2013;63:15-22. http://dx.doi.org/10.1016/j.apsoil.2012.09.001.

[17] Donate-Correa J, Leon-Barrios M, Perez-Galdona R. Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. Plant Soil. 2005;266:261-272. DOI: 10.1007/s11104-005-0754-5.

[18] Khan AL, Halo BA, Elyassi A, Ali S, Al-Hosni K, Hussain J, Al-Harrasi A, Lee IJ. Indole acetic acid and ACC deaminase fromendophytic bacteria improves the growth of *Solanum lycopersicum*. Electronic J Biotechnol. 2016;21:58-64. http://dx.doi.org/10.1016/j.ejbt.2016.02.001.

[19] Ahmad Z, Wu J, Chen L, Dong W. Isolated *Bacillus subtilis* strain 330-2 and its antagonistic genes identified by the removing PCR. Sci Rep. 2017;7(1):1777. DOI: 10.1038/s41598-017-01940-9.

[20] Ren JH, Li H, Wang YF, Ye JR, Yan AQ, Wu XQ. Biocontrol potential of an endophytic *Bacillus pumilus* JK-SX001 against poplar canker. Biological Control. 2013;67(3):421-430. DOI: 10.1016/j.biocontrol.2013.09.012.

[21] Collins DP, Jacobsen BJ. Optimizing a *Bacillus subtilis* isolate for biological control of sugar beet cercospora leaf spot. Biological Control. 2003;26(2):153-161. DOI: 10.1016/s1049-9644(02)00132-9.

[22] Kumar P, Dubey RC, Maheshwari DK. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res. 2012;167(8):493-499. DOI: 10.1016/j.micres.2012.05.002.

[23] Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda Mdel C, Glick BR. Plant growth-promoting bacterial endophytes. Microbiol Res. 2016;183:92-99. DOI: 10.1016/j.micres.2015.11.008.

[24] Krawczyk K, Zwolińska A, Kamasa J, Maćkowiak-Sochacka A, Przemieniecki S. Identification and characterization of plant growth promoting endophytic bacteria. Progress Plant Protect. 2016;56:100-109. DOI: 10.14199/ppp-2016-018.

[25] Devi KA, Pandey G, Rawat AKS, Sharma GD, Pandey P. The Endophytic Symbiont – Pseudomonas aeruginosa Stimulates the Antioxidant Activity and Growth of Achyranthes aspera L. Front Microbiol. 2017;8:1897. DOI: 10.3389/fmicb.2017.01897.

[26] Zhao L, Xu Y, Lai XH, Shan C, Deng Z, Ji Y. Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. Braz J Microbiol. 2015;46(4):977-989. DOI: 10.1590/S1517-838246420140024.

[27] Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 Biotech. 2016;6(1):60. DOI: 10.1007/s13205-016-0393-y.

[28] Ji SH, Gururani MA, Chun SC. Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. Microbiol Res. 2014;169(1):83-98. DOI: 10.1016/j.micres.2013.06.003.

[29] Starovic M, Josic D, Pavlovic S, Drazic S, Postic D, Popovic T, Stojanovic S. The effect of IAA producing *Bacillus* sp. Q3 strain on marshmallow seed germination. Bulg J Agric Sci. 2013;19:572-577. https://www.researchgate.net/publication/258820995_The_effect_of_IAA_producIng_Bacillus_sp_Q3_strain_on_marshmallow_seed_germination.

PRODUKCJA KWASU INDOLILOOCTOWEGO PRZEZ ENDOFITYCZNE SZCZEPY *BACILLUS*

Samodzielna Katedra Biotechnologii i Biologii Molekularnej
Uniwersytet Opolski, Opole

Abstrakt: Celem pracy było wyizolowanie i scharakteryzowanie bakterii endofitycznych korzeni buraka cukrowego pod kątem ich zdolności do *produkcji kwasu indolilooctowego* (IAA). Materiał doświadczalny do izolacji bakterii endofitycznych stanowiły korzenie burak cukrowego. Ilość wytworzonego IAA przez endofityczne szczepy *Bacillus* (*B. amyloliquefaciens*, *B. megaterium*, *B. subtilis*) oznaczono metodą kolorymetryczną z odczynnikiem Salkowskiego. Szczepy hodowano w pożywce LB (Laurin Broth) suplementowanej L-tryptofanem w stężeniach 100–10000 μg/cm^3 i inkubowano w 30 ^{o}C przez 7 dni. Najwyższe stężenie IAA odnotowano po 4 dniach w hodowlach zawierających 10000 μg/cm^3 L-tryptofanu. Dla szczepu *B. subtilis* i *B. megaterium* stężenie IAA wynosiło około 82,00 μg/cm^3, a dla szczepu *B. amyloliquefaciens* 127,28 μg/cm^3. Badane szczepy *Bacillus* spp. wytwarzały IAA na różnym poziomie zarówno w obecności L-tryptofanu jak i przy jego braku.

Słowa kluczowe: *Bacillus,* endofity, tryptofan, auksyny